

Performance study of lab scale algae production-harvesting integrated system (APHIS) in improving algae oil for sustainable energy

Mohd Azrin Mohd Said^{1,*}, Ahmad Shazrin Mohd Ghazali¹, Melvin Sean Joseph¹, Abu Saleh Ahmed¹, M. Shahidul Islam¹, Abdullah Yassin¹, Hishammudin Afifi Huspi¹, Ibrahim Yakub²

¹Universiti Malaysia Sarawak, Faculty of Engineering, Department of Mechanical and Manufacturing Engineering, 94300 Kota Samarahan, Malaysia

²Universiti Malaysia Sarawak, Faculty of Engineering, Department of Chemical Engineering and Energy Sustainability, 94300 Kota Samarahan, Malaysia

Abstract: Due to the decreasing of fossil fuel, the new method to replace the shortage of this energy are been studied by many researcher. Renewable energy from the usage of nature is one of interesting part nowadays. Biodiesel is one the example of renewable energy. There are many ways or raw material to produce biodiesel. One of the examples is Algae. Algae offer several advantages over other terrestrial plants as alternative biofuel including their rapid growth rate, greenhouse gas fixation ability, and high production capacity of lipids. Therefore in this study, a lab-scale Algae Production Harvesting Integrated System (APHIS) was designed and built to produce the algae for biodiesel production. The objectives of this APHIS are to cultivate the algae or microalgae using photo bioreactor concept and then harvest the algae using ultrafiltration (UF) membrane in one closed loop systems. The methodology that used in this study was using cell counting of production and mechanical harvesting using membrane. The marine-type microalgae, *Nannochloropsis* sp. were selected, cultured and maintained in air-conditioned room. The result of growth rate of *Nannochloropsis* sp. was monitored for three salinity concentrations (26 ppt, 28 ppt and 30 ppt). UF membrane has been proved to be a useful laboratory tool for the concentration of *Nannochloropsis* sp. APHIS was capable of achieving 75% biomass recovery when the volume concentration factor (VCF) was 10. In addition, algae recovery was highly correlated to the volume concentration factor. Others factors which affect the performance of membrane filtration for algae harvesting also been studied. In future, this lab scale APHIS can be used as laboratory experiment or education to student and also as kick start of production of biodiesel.

Key words: Performance; Microalgae; Lab scale; Production; Harvesting; Energy; Biodiesel

1. Introduction

Nowadays, many study shown that the overwhelming rates of fuel consumption at present may cause the world fossil fuel reserves will be depleted in less than 50 year (Rodolfi et al., 2008). With the increase in GHG emissions, which mainly cause by the large scale use of fossil fuels for transport, electricity and thermal energy generation, it has become increasingly imperative to develop abatement methods and adopt policies to minimise impacts of global warming (Brennan & Owende, 2010).

Thus, the research and exploration for alternative energy sources such as wind, solar and biofuel are now more vital than ever. Biofuels are considered to be a desirable alternative because they are "greener" and sustainable. The recent research of the use of an alternative, non-food related feedstock such as oil from algae has received growing interest in global scale. Mata, Martins, and Caetano (2010) claimed that although the growing popularity in the research, it is still in its early stage. Even solid waste can be

one of the potential of renewable energy (Azrin and Bush, 2014). As study done by Firoz et al. (2012), microalgae have received substantial interest as a potential feedstock for biofuel production because they can produce useful quantities of polysaccharides (sugars) and triacylglycerides (TAGs) and seem to be most feasible alternative energy resource for biofuel production overcoming the disadvantages of first and second generation biodiesels. However, Firoz et al. (2012) stated that commercialization of this technology should have several key performance features including low energy consumption; complete recycling of water and nutrients, and no addition of harmful chemicals/materials.

Algae are recognized as one of the oldest life-forms (Falkowski & Raven, 1997). Others research by Dragone, Fernandes, Vicente and Teixeira (2010), they stated that as the cells grow in aqueous suspension, they have more efficient access to water, CO₂, and other nutrients. Generally, microalgae are classified in accordance with their colors. The most important classes are: green algae (Chlorophyta),

* Corresponding Author.

red algae (Rhodophyta) and diatoms (Bacillariophyta).

In the earth ecosystem, diatoms microalgae are probably the largest group of biomass producers as they are the dominant life form in phytoplankton. Besides that, diatoms contain high oil content as they are capable of accumulating oils in their unicellular structure (Brennan, 2010). Green algae such as *Chlorella Vulgaris* can be found abundantly in fresh water. Microalgae are present in all existing earth ecosystems, not just aquatic but also terrestrial and they have many species living in a wide range of environmental conditions. It is estimated that more than 50,000 species exist, but only a limited number, of around 30,000, have been studied and analyzed (Richmond, 2004).

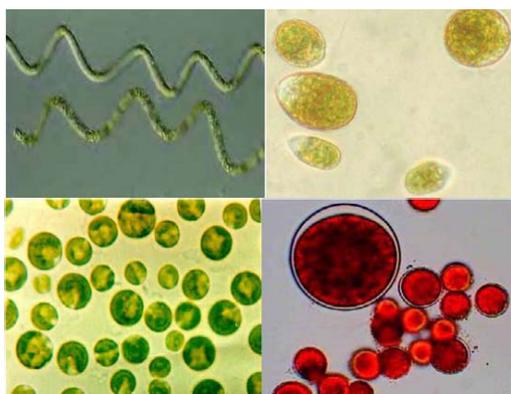


Fig. 1: Micrographs of Cultivated Algae Species (Lundquist et al., 2010)

Fig. 1 shows the micrographs of cultivated algae species. From top left, *Spirulina* sp (*Arthrospira platensis*) and from top right, *Dunaliella Salina* sp. Bottom left, *Chlorella vulgaris* sp while bottom right, *Haematococcus pluvialis* sp. Microalgae reproduce themselves using photosynthesis to convert sun energy into chemical energy, undergoing a complete growth cycle every few days and they can grow almost anywhere in wet conditions.

It can be said that several species of microalgae can have oil contents up to 80% of their dry body weight. In addition, some microalgae can double their biomasses within 24 hours and the shortest doubling time during their growth is about 3.5 hours making them an ideal renewable source for biofuel production (Chisti, 2007).

There are also several challenges remain in the development of algae production and harvesting system. The two major challenges in implementing an integrated algae production and harvesting system include large-scale production of algae and algae harvesting it in a way that allows the downstream processing to produce biofuels and other by-products of value. The challenges in terms of large-scale production of algae include (Christenson, 2011):

1. Nutrient supply and recycling
2. Gas transfer and exchange
3. Photo synthetically active radiation (PAR) delivery
4. Culture integrity

5. Environmental control

6. Land and water availability

7. Harvesting

The most pressing issue was not in the production system, but in the harvesting and downstream processing of algae in a way that suitable for the production of bioproducts such as biodiesel (Uduman, Qi, Danquah, Forde, & Hoadley, 2010). For harvesting part, according to the rule of thumb used by MacKay and Salusbury (1988), for large scale production (>20,000l), centrifugation may be more attractive, whereas at small scale (<2,000l), cross-flow microfiltration may be a better choice.

Therefore, this study focuses on the application and analysis of an integrated system for the production and harvesting microalgae in one closed-loop system. Therefore the performance study of algae production harvesting integrated system (APHIS) in lab scale is referring to the capacity or size of production of this APHIS. While further studies and research need to be done, if successful, algae-based fuels can help meet the world's energy growing demand especially for transportation fuel while reducing greenhouse gases (GHG) emissions.

2. Methodology

This study was conducted in Fluids Lab, Faculty of Engineering and University Malaysia Sarawak (UNIMAS). The performance analysis of Lab Scale (APHIS) will only covered volume used for harvesting is less than 10 gallon or it can be approximately 37.85 litres.

As lab scale APHIS falls under the category of closed reactors. APHIS design attempts to balance the benefits of low cost open ponds with the control of closed system. This is achieved by placing a cover over the cultivation tank. In terms of harvesting approach, membrane technology is selected based on ultrafiltration systems due to the absence of chemical additives, and able to function at moderate temperature and pressure. Besides that, cross flow filtration method has a few advantages over other recovery techniques such as conventional filtration, centrifugation, flocculation and sedimentation such as better filtration rates can be achieved to completely remove debris and microalgae cells. Microalgae harvesting using APHIS was performed batch-wise where microalgae suspension are added into the system per batch and harvested. Then permeate, which is water produced from the filtration process can be used again for next cultivation. Hence, the system is able to work on a closed-loop cycle.

2.1. APHIS Design

The APHIS design was been done using Design Matrix. The final design has been selected and fabricated. Fig. 2 shows the final product fabrication of the final APHIS.

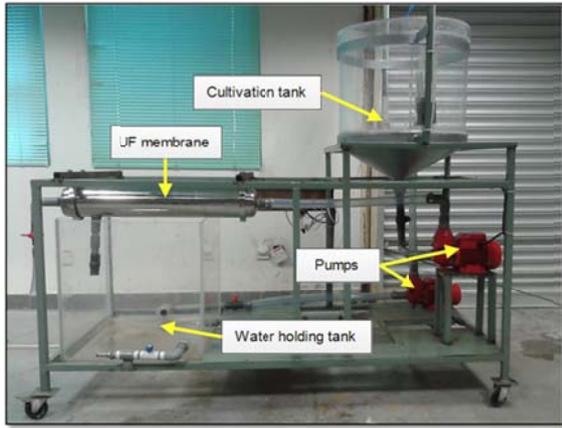


Fig. 2: Final design fabrication of APHIS (Azrin et al., 2015)

2.2. APHIS setup

The components of APHIS are cultivation tank, centrifugal pumps, water tank, piping system, light bulbs and ultrafiltration (UF) membrane. Fig. 3 shows the schematic flow diagram of APHIS. The APHIS also equipped with an air pump to induce mixing during cultivation process.

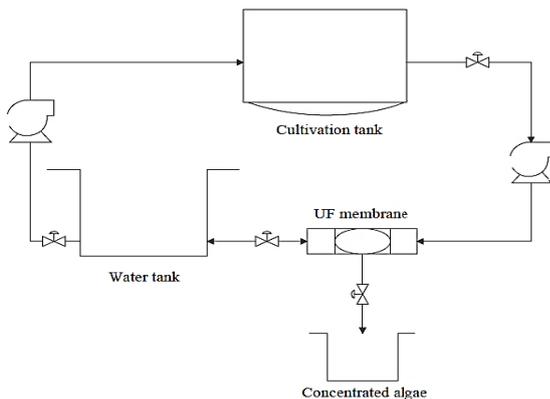


Fig. 3: Schematic diagram of APHIS

2.3. Microalgae suspension

The microalgae feed, *Nannochloropsis* sp., used in this study was collected from Fisheries Research Institute (FRI) Malaysia Sarawak, Santubong. *Nannochloropsis* sp. is a unicellular, of about 2–5 µm cell size golden green algae with a salinity ranges from 25-30 ppt (Rodolfi et al., 2008). The marine microalgae were found to be suitable as raw materials for biofuel production due to its high oil content of 28.7% (Gouveia & Oliveira, 2009). Conway medium was used to promote algae growth. The culture medium was prepared from seawater and an equivalent dosage of the following Conway solution as show in Table 1:

Table 1: Procedure for medium preparation

Solution	<i>Nannochloropsis</i> sp
A	1.0-ml
C	0.1-ml
The above dosage is for 1-L of culture water.	

2.4. Production and culture conditions

The production cultures start in the test tube obtained from the FRI and scale-up into subsequent culture of 250-ml Erlenmeyer flask, 1-L Erlenmeyer flask, 20-L squared-container and cylindrical tank of APHIS. The culture was grown in the Fluids Laboratory of UNIMAS. Fig. 4 shows the flow chart of culture. The stock culture in the test tube was cultivated in 250-ml conical flasks for 4-7 days to allow the algae to increase its density. Then, the culture was sub-cultured in the 1-L conical flasks for another 4 days and transferred to the 20-L container before harvesting. The culture temperature was maintained in the ranges between 22 to 25 °C for entire culture period. Fluorescent bulbs were used to provide light emission for 24-hours period. Continuous aeration was provided using air pump to induce mixing and increasing the area of contact of culture between the air and medium. Fig. 3.2 shows the flowchart for scale-up process of *Nannochloropsis* sp. For this study, the volume used for harvesting is less than 10 gallon (approximately 37.85 litres) for lab scale study.

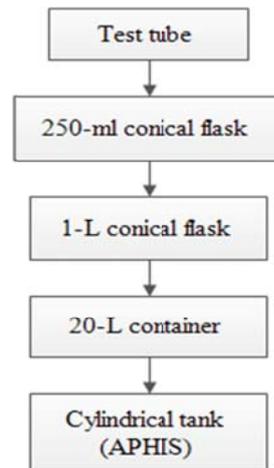


Fig. 4: Flowchart for *Nannochloropsis* sp. culture

2.5. Cell counting

For salinity study, the experiment was carried out at the laboratory at FRI. To monitor the growth rate of microalgae, the salinity of culture medium was set at three different concentrations which are 26 ppt, 28 ppt, and 30 ppt. Salts were added into the medium until the desired salinities were reached. Experiments were carried out in 2-L Erlenmeyer flasks each containing 1500 ml of appropriate medium. All cultures were maintained in an air conditioned room and kept at continuous illumination and aeration. The experiments were set up for 10 days. Subsamples were drawn daily from each culture medium and the cell count was determined by using a Neubauer haemocytometer and light microscopy to measure the cell density. Growth curves for each culture media was prepared by plotting the average cell density vs corresponding cultivation time using EXCEL computer program. The best growth phase for *Nannochloropsis* sp. was

then selected and used as inoculum for mass culture and to perform a cross flow filtration using APHIS.

2.6. Microalgae harvesting

Fresh culture microalgae were harvested using ultrafiltration (UF) membrane after sufficient growth. During experimentation, 20-L of microalgae suspension from the cultivation tank is pumped through the filtration module. The volume of permeate (water) and retentive (concentrated microalgae) from the process were recorded for each cycle. The permeate (water) from the filtration process is channel to the water holding tank which can be used again for next batch of fresh culture. The concentrated microalgae can be collected at the drainage point.

2.7. Analytical techniques

Biomass concentration was evaluated by dry weight measurements. Dry weight is the most common method to evaluate the quantity of organic matter in a sample. In order to determine the dry weight of microalgae, the microalgae biomass was dried using forced convection oven for about 4 hours at 60°C and the weight of biomass was determined gravimetrically (g/l). All data were collected to produce the preliminary data for APHIS.

For UF membrane, the criteria assessments of membrane performance were volume concentration factor (VCF) and biomass recovery (BR). Volume concentration factor is defined as the ratio between initial (V_o) and final (V_t) volumes of the culture before and after the concentration process, respectively as shown in Equation 1:

$$VCF = V_o / V_t \quad (1)$$

Biomass recovery can be defined as the percentage of the biomass that can be recovered from the feed as shown in Equation 2:

$$BR = (V_t C_t) / (V_o C_o) \times 100\% \quad (2)$$

Where C_o and C_t are the initial biomass (in grams per liter) before concentration and final biomass (in grams per liter).after the concentration, respectively.

2.8. Membrane filtration

The membrane used in this study was hollow fibre UF membrane with a mean pore size of 0.1 μ m. According to the supplier, the membrane consists of 13,000 pieces of capillary in a single module and a flow rates range from 1500-2000 litres per hour. APHIS has a current membrane length of 0.67 meter and a diameter of 0.12 meter. The membrane has an effective area of 0.2752 m^2 . During filtration, the pressure was kept constant.

3. Results and discussions

Membrane filtration was selected for APHIS because the cross flow or tangential filtration offers several advantages over other harvesting methods

such as it can achieve complete removal of algae from the culture medium while retaining residual nutrients. Thus, the microalgae medium can be recycled for next cultivation. This principle is vital in integrating the production and harvesting system of APHIS. In addition, membrane filtration does not require additives or coagulant and able to perform under moderate pressure and temperature. Besides that, the design of APHIS was also the limiting factor when choosing the harvesting method. The most suitable choice would be membrane filtration as the UF membrane can be fit into the frame of APHIS and perform harvesting process without any difficulty.

3.1. Growth Rate

The marine-type algae, *Nannochloropsis* sp. was cultured in Conway medium for a period of 10 days in the 2-L flasks as shown in Fig. 5. Varying salinity levels were given to check for the difference in cell growth for all three concentration of salinity. To avoid variations, the experiments were performed under the same growth conditions of temperature, light and nutrient intake

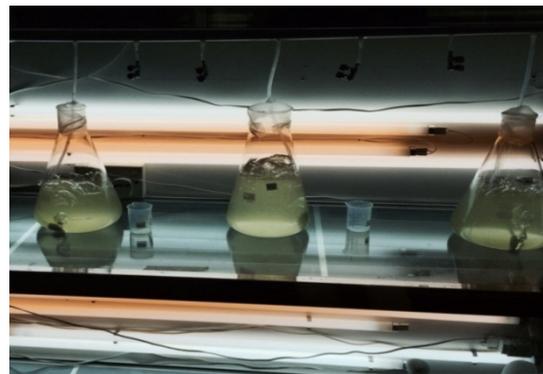


Fig. 5: Cultivation of *Nannochloropsis* sp.

The graph in Fig. 6 shows the growth curve for *Nannochloropsis* sp. in a period of 10 days. The cell count was taken every day to produce the growth curve for all three salinity levels.

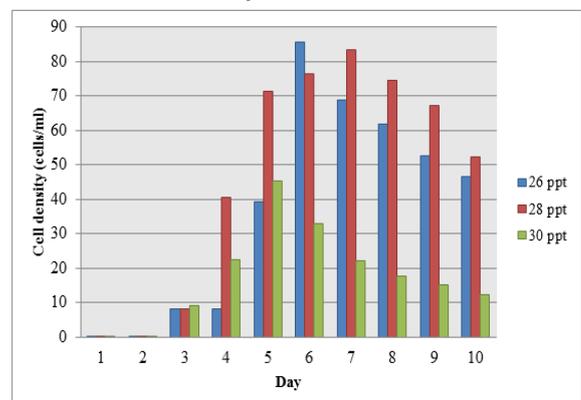


Fig. 6: Cell density of *Nannochloropsis* sp. with response to different salinity concentrations

As seen in Fig. 6, in case of 26 parts per thousand (ppt), the cell growth increases from day 1 to day 6 with the maximum cell density of 85.65 cells/ml. Then, a decrease in cell growth was recorded until

day 10. For 28 ppt, the cell growth showed a sudden increase on day 4 (40.65 cells/ml) and gradually increased until day 7 with maximum count of 83.30 cells/ml. In case of 30 ppt, the maximum cell growth of 45.3 cells/ml was recorded on day 5 and gradually decreased until day 10. The cell count for 30 ppt showed the lowest growth rate, whereas the cell count for 26 ppt and 28 ppt showed a higher growth rate and quite similar trend. For all three salinity concentrations, the cell density for the first three days was relatively low due to the low inoculation ratio or small number of cells is added to the new media. The cell density increases from day 4 onwards, followed by a gradual decreased until day 10 when the declining phase started. The reason for this is because the nutrient needed for cell growth was nearly finished, thus inhibiting the cell growth.

Therefore, salinity of either 26 ppt or 28 ppt was selected as inoculum for mass culture for harvesting purpose using APHIS

3.2. Microalgae biomass

APHIS operated in batch-wise. In batch operation, no feed is introduced during the filtration process. Therefore, over time and due to permeation, the culture volume in the feed tank decreases and due to the partial or complete retention of biomass, its concentration increases as shown in Fig. 7.

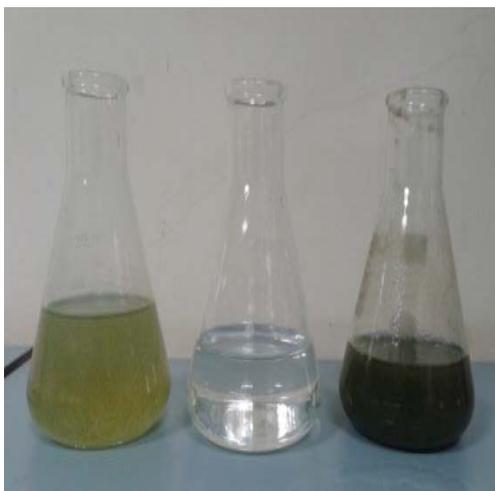


Fig. 7: Initial suspension, permeate (water) and concentrated microalgae

Besides that, the color of the culture was observed to be darker in the final concentrated microalgae compare to the initial suspension. At the higher concentrations, the biomass is a paste or slurry containing $\geq 2-7\%$ total suspended solid, corresponding to a 100–200 volumetric concentration factor (Bilad et al., 2014).

To assess the relationship between the recovery of algal biomass and volume concentration factor, the recovery of three concentration factors (VCF=10, 20, 33) was determined and the initial biomass was 0.2 g/L. The initial and final volumes of culture were recorded for three trials and the volumetric

concentration factor was determined, and the results were shown in Table 2.

Table 2: Volume and concentration measurement

Trial	Initial volume (L)	Final volume (L)	Initial concentration (g/L)	Final concentration (g/L)
1	20	2	0.2	1.5
2	20	1	0.2	1.79
3	20	0.6	0.2	1.87

Using the tabulated data and calculated with Eq. (1) and Eq. (2) the volume concentration factor and biomass recovery for the three trials were calculated as shown in Table 3.

Table 3: Biomass recovery of different VCFs

VCF	Biomass recovery (%)
10	75
20	44.8
33	28

In Fig. 8, the results showed that the biomass recovery for the examined (28%) was achieved at a high concentration factor (VCF=33). This occurs due to the fouling phenomenon aggravated in the filtration module. However, the biomass recovery for (VCF=10) and (VCF=20) were found to be 75% and 44.8%, respectively. Compared with the group (VCF=10), an increase to (VCF=20) did cause additional biomass loss of about 30.2%. Further accumulation of culture medium corresponding to VCF=33 increased biomass losses to 72%. Hence, it should be pointed out that the volume concentration factor was a significant influencing factor on the *Nannochloropsis* sp. concentration. Besides that, it can be concluded that a volume concentration factor of 10 was necessary to produce a biomass recovery higher than 75%.

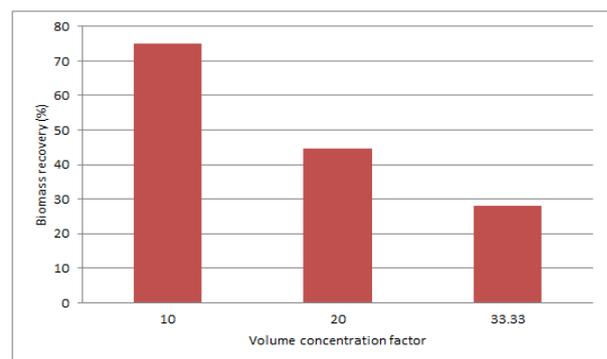


Fig. 8: Effect of VCF on BR

APHIS was capable of producing a biomass recovery of 75% with the concentration factor of 10. A study by Huang et al. (2011), presented that a volume concentration factor of 10 was necessary to guarantee a biomass recovery higher than 90%. To conclude, it was found that *Nannochloropsis* sp. could be concentrated with high recovery in a lab-scale experiment.

3.3. Performance comparison

Table 4 and Table 5 are the results of comparison for APHIS with other technologies in terms of production and harvesting system. APHIS operated using a closed-reactor type tank for algae cultivation which was equipped with three light bulbs for light emission. The cultivation tank was placed with a top cover to minimize any contamination risk which can affect the growth of algae. The majority of open pond system has a paddlewheel or air pump to provide mixing and recirculation, but they require a large land.

Table 4: Comparison of algae production approaches

Production approach	Maximum volume capacity	Area requirement (m ²)	Contamination risk	Evaporation
APHIS	500-L	3.6	+	+
Open pond	100 ton	250	++++	++++
Closed pond	100 ton	250	+++	++
Tubular system	100 ton	1200	+	++
Plastic bag system	100 ton	1200	+	++
Pyramid PBR system	100 ton	60	+	+

Note: '+' represent the level of operating characteristic.

Table 5: Comparison of algae harvesting method

Harvesting method	Recovery	Scale	Reference
Ultrafiltration membrane	75%	Lab	This study
Centrifugation	>90%	Bench	Shen et al. (2009)
Sedimentation	10-90%	Pilot	Shen et al. (2009)
Dissolved air floatation	50-90%	Pilot	Uduman et al. (2010), Shen et al. (2009)
Spool harvester	70-85%	Bench	Christenson L. (2011)

Therefore this study shows that for lab scale APHIS was good integrated system with its own limitations in order to promote renewable and sustainable energy for future.

4. Conclusion

The results of this study fulfilled the aims of research. APHIS design was using closed loop system using photobioreactor concept for cultivation and ultrafiltration membrane for algae harvesting as it offers several advantages over other harvesting methods. This includes it can achieve complete removal of algae from the culture medium while retaining residual nutrients, does not require additives or coagulant and able to perform under moderate pressure and temperature.

Marine-type algae, *Nannochloropsis* sp was selected and cultured in a closed-environment room and maintained in an air conditioned room. Continuous light emission and mixing were provided to promote growth. The growth rate of *Nannochloropsis* sp for three different salinity concentrations (26 ppt, 28, ppt and 30 ppt) were

evaluated. The cell count for 30 ppt showed the lowest growth rate while the cell count for 26 ppt and 28 ppt showed a higher growth rate and quite similar trend. Thus, the salinity of either 26 ppt or 28 ppt was selected as inoculum for mass culture for harvesting. An overall algae biomass recovery of 75% was achieved when the volume concentration factor was 10. In other words, algae recovery was highly correlated to the initial the volume concentration factor. A high volume concentration factor resulted in a low biomass recovery. APHIS was capable of producing a biomass recovery of 75%. The values in this study represent a preliminary data for lab-scale APHIS and a promising baseline for future improvements such as full-scale application. Results of this study indicate that the lab scale APHIS represents a promising approach to the production and harvesting algae in an integrated system.

5. Acknowledgment

The author wishes to extend their gratitude to the Ministry of Education (MOE), Malaysia and University Malaysia Sarawak (UNIMAS) and RIMC UNIMAS for providing financial support under the research Grant Scheme No: RAGS/TK/01(03)/936/2012(37). Many thanks to all the colleagues, undergraduate students from University Malaysia Sarawak for assistance and involve in this research project.

References

- Bilad M.R., Hassan A. A., I. Vankelecom F.J. (2014). Membrane technology in microalgae cultivation and harvesting: a review. *Biotechnology Advances* 32:1283-1300.
- Brennan, L. & Owende, P. (2010). Biofuels from microalgae: A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews* 14:557-577
- Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances*, 25:294-306.
- Christenson L. (2011). Algal biofilm production and harvesting system for wastewater treatment with bio-fuels by-products. All graduate theses and dissertations. Paper 994. <http://digitalcommon.usu.edu/etd/994>.
- Dragone, G., Fernandes, B., Vicente, A.A. & Teixeira, J.A. (2010), Third generation biofuels from microalgae in current research, technology and education topics in applied microbiology and microbial biotechnology, Mendez-Vilas A (ed.). *Formatex*, 1355-1366.
- Falkowski P.G. & Raven J.A. (1997). *Aquatic photosynthesis*. London: Blackwater Science; pg. 375.

- Firoz, A., Abhijit D., Roesfiansjah, R., Saleh, M., Hazim, M., & Abdul, B. (2012). Biofuel from algae- is it a viable alternative. *Procedia engineering* 49: 221-227.
- Gouveia L. & Oliveira A.C. (2009). Microalgae as a raw material for biofuel production. *Microbiol Biotechnol* 36:74-296.
- Lundquist T.J., Woertz I.C., Quinn N.W.T., & Benemann J.R. (2010). A realistic technology and engineering assessment of algae biofuel production. University of California: Berkley, California.
- M.S.M. Azrin and E.R. Bush (2014). A Study of Solid Waste Generation in Residential College UNIMAS West Campus as Potential Renewable Energy. *Journal of Applied Science & Process Engineering (JASPE)* Vol. 1, No. 1, 20. e-ISSN 2289-7771 pg. 45-50.
- M.S.M. Azrin, M.G.A. Shazrin, M.S. Joseph, A.S. Ahmed, M. S. Islam, A. Yassin, H.A Huspi, I. Yakub., (2015). Development of Algae Production-Harvesting Integrated System for Future Sustainable Energy. *Aust. J. Basic & Appl. Sci.*, 7(13): x-x, 2015. in press.
- MacKay, D. & Salusbury, T. (1988) Choosing between centrifugation and cross flow microfiltration, *Chem. Eng.*, 477, 45-50.
- Mata T.M., Martins A.A., & Caetano N.S. (2010). Microalgae for biodiesel production and other applications: a review. *Renew Sustain Energy Rev* 14:217-232.
- Richmond A. (2004). *Handbook of microalgal culture: biotechnology and applied phycology.* Blackwell Science Ltd.
- Rodolfi, L., Zittelli, G. C., Bassi, N., Padovani, G., Biondi, N., Bonini, G., & Tredici, M.R. (2008). Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering*, 102(1), pg 100.
- Shen Y., Yuan Y., Pei Z.J., Wu Q. & Mao E. (2009). Microalgae mass production methods. *Trans ASABE*, 52:1275-1287.
- Uduman N, Qi Y, Danquah MK, Forde GM, & Hoadley A. (2010). Dewatering of microalgal cultures a major bottle neck to algae-based fuels. *J Ren Sust Energy* 2.